

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Jaime A. Rabi	Confirmation No.:	1836
Serial No.:	10/806,296	Art Unit:	1623
Filed:	March 22, 2004	Examiner:	Krishnan, Ganapathy
For:	Methods of manufacture of 2'-deoxy-beta-L-nucleosides	Attorney Docket No:	11874-010-999

**DECLARATION UNDER 37 C.F.R. 1.132**

Mail Stop Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

1. I have about forty years of experience in the discovery, research and development of pharmaceutical agents, including deoxynucleoside antiviral agents.
2. I received my Ph.D. degree in chemistry from Washington University, St. Louis, and subsequently completed postdoctoral research at the Sloan-Kettering Institute for Cancer Research, Rye, New York.
3. I served as an assistant professor in the Department of Biochemistry at the School of Pharmaceutical Sciences of the University of Chile in Santiago from 1966 to 1967.
4. I served as a teaching assistant at the Department of Chemistry at Washington University from 1967 to 1969.

5. I served as a research assistant at the Department of Chemistry at Washington University from 1969 to 1971.
6. I served as a post-doctoral fellow at the Sloan-Kettering Institute for Cancer Research (Cornell University) in Rye, New York from 1971 to 1972.
7. I served as an associate professor at the Natural Products Research Center -NPPN of the Federal University of Rio de Janeiro from 1973 to 1994.
8. I served as a researcher (I-A, highest level) of the National Brazilian Research Council (CNPq) from 1976 to 1989.
9. I served as the Director of the Natural Products Research Center of the Federal University of Rio de Janeiro from 1989 to 1992.
10. I have served as the Managing Director and Director for Research and Development at Microbiologica Quimica e Farmaceutica (“Microbiologica”) since 1994.
11. I served as a member of the Board of Directors of Pharmasset, Atlanta, Georgia from 1998 to 2004.
12. I am a member of the International Society for Nucleosides, Nucleotides and Nucleic Acids and the International Society for Antiviral Research.
13. I served as a consultant to the Ministry of Science and Technology of the Brazilian Government from 1996 to 2001.
14. I am currently a partner and CEO at Microbiologica. I joined Microbiologica as a consultant in September 1983. Microbiologica and I have a financial interest in the above-referenced patent application.

15. I am a named inventor in the above-referenced patent application.
16. I have read and understood the Office Action dated September 2, 2008 ("Office Action") for the above-referenced application.
17. I have read and understood the references cited by the Examiner in the Office Action.
18. In response to the rejection under 35 USC §103(a) stated in the Office Action, experiments were conducted to generate additional data to show that the processes of the currently amended claims provide unexpected and synthetically relevant results as discussed in paragraphs 21-22 and 23-24 below.
19. As shown below, a number of experiments for coupling  $\alpha$ -1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose with silylated thymine in different solvents ("the Experiments") were performed by me or under my direction according to the following general procedure:

After a reactor (2 L) was flushed with nitrogen for 10 minutes, thymine (41.8 g, 0.332 mole), ammonium sulfate (0.67 g), 1,1,1,3,3,3-hexamethyldisilazane (HMDS) (75 mL), and toluene (42 mL) were charged to the reactor. The reaction mixture was stirred and heated for about 5-8 hours to a gentle reflux. The reaction mixture was then cooled to about 60 °C and distilled under vacuum to remove excess 1,1,1,3,3,3-hexamethyldisilazane and toluene. After all HMDS was gone, the reaction mixture was cooled to about 60 °C and then a solvent (10 parts by volume based on the amount of the 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose) was selected and charged to the reaction mixture under nitrogen. After the solution

was stirred and cooled to about 22 °C, 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose (122.7 g, 0.316 mole) was charged with rapid, portionwise addition. The reaction mixture was stirred for about 2.5 hours. After the 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose was no longer visible by TLC, the reaction mixture was stirred for 1 more hour at about 22 °C. An HPLC analysis was conducted on the reaction mixture to determine the ratio of the  $\beta$  to  $\alpha$  isomer, which are reported in the tables below.

20. The following results were obtained as described above but using different solvents:

Solvent	Ratio of Protected $\beta$ -L-2'-deoxythymine to Protected $\alpha$ -L-2'-deoxythymine measured immediately after the coupling reaction	Dielectric Constant <sup>#</sup>
Chloroform	10 : 1	4.81 at 20 °C
Acetonitrile	<1 : 1	37.5 at 20 °C
Dichloromethane	4 : 1	8.93 at 25 °C
Ethyl Acetate	1.1 : 1	6.02 at 25 °C
Methyl Isobutyl Ketone	1.1 : 1	13.11 at 25 °C
N,N-Dimethylformamide	<1 : 1	36.71 at 25 °C

Note: <sup>#</sup> The dielectric constant values were obtained from the website at <http://macro.lsu.edu/howto/solvents/Dielectric%20Constant%20.htm>. The ratio of silylated thymine to 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose was 1.05. The volume of the solvent was always 10 parts, based on the amount of the 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose.


21. The Experiments were repeated in chloroform at different ratios of the silylated thymine to the 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose, and the following results were obtained :

Ratio of silylated thymine to the 1- $\alpha$ -chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose	Ratio of Protected $\beta$ -L-2'-deoxythymidine to Protected $\alpha$ -L-2'-deoxythymidine
1.05 : 1	9-11 : 1
1.10 : 1	16-18 : 1
1.30 : 1	20-30 : 1
2.00 : 1 <sup>#</sup>	46-48 : 1

Note: <sup>#</sup> This experiment was repeated in acetonitrile and the ratio of the  $\beta$  to  $\alpha$  isomer was found to be  $<2 : 1$ .

22. I personally ran many of the experiments, led a group of technicians working on this project, and also supervised teams in scaling up the synthesis at Microbiologica and elsewhere including organizations in the United States and Europe.
23. It is both unexpected and surprising that chloroform provided much higher stereoselectivity than acetonitrile, which is the only solvent disclosed in the cited references, *i.e.*, Gosselin *et al.* (U.S. Patent No. 6,444,652) and Weis *et al.* (WO 96/13512), for allegedly similar coupling reactions. When the coupling reaction was run at a silylated thymine to 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose ratio of 1.05:1, the  $\beta$  to  $\alpha$  ratios in chloroform and acetonitrile are respectively 10:1 and  $<1:1$ . When the coupling reaction was run at a silylated thymine to 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose ratio of 2.00:1, the  $\beta$  to  $\alpha$  ratios in chloroform and acetonitrile are respectively 46-48:1 and  $<2:1$ .
24. It is also both unexpected and surprising that other solvents such as dichloromethane, ethyl acetate, methyl isobutyl ketone and N,N-dimethylformamide provide much lower stereoselectivity than chloroform. When the coupling reaction was run at a silylated thymine to 1-chloro-2-deoxy-3,5-di-O-toluoyl-L-ribose ratio of 1.05:1, the  $\beta$  to  $\alpha$  ratios in chloroform, dichloromethane, ethyl acetate, methyl isobutyl ketone and N,N-dimethylformamide are respectively 10:1, 4:1, 1.1:1, 1.1:1 and  $<1:1$ .

25. I, Jaime A. Rabi further declare that all statements made herein are of my own knowledge to be true and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of this application or any patent that may issue there from.



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JAIME A. RABI

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16 February, 2009.